Breast Cancer RESEARCH

VIEWPOINT

New insights into signalling networks regulating breast cancer stem cells

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Abstract

In a recent paper, Aceto and colleagues report that Src homology 2 domain-containing protein tyrosine phosphatase 2 (Shp2) plays a critical role in maintenance of breast tumour-initiating cells, and they define novel effectors downstream of Shp2 that regulate cellular invasion and self-renewal, including the transcription factors c-Myc and zinc finger E-box binding homeobox 1 and the suppressor of miRNA biogenesis lin-28 homolog B. These findings provide important mechanistic insights into breast tumourigenesis and highlight Shp2 as a potential therapeutic target.

The cancer stem cell (CSC) hypothesis proposes that certain malignancies, including breast cancer, are driven by a population of CSCs that are tumorigenic and capable of self-renewal and differentiation into nontumorigenic cells. Tumour-initiating cells (TICs) are operationally defined as cells that seed tumours when transplanted in mice [1]. The highlighted study addresses the role of Src homology 2 domain-containing protein tyrosine phosphatase 2 (Shp2) in maintenance of the CSC/TIC subpopulation [2]. In breast cancer, signalling via this phosphatase can be amplified via upregulation of particular receptor tyrosine kinases, such as erbB2 (also known as HER2) or c-Met [3,4], or via overexpression of the docking protein growth factor receptor bound 2-associated binder 2 [5-8]. From a signalling perspective, Shp2 is required for maximal and/or sustained activation of the Ras/Erk pathway by a variety of growth factor and cytokine receptors, but it also regulates other pathways, including phosphatidylinositol 3-kinase/Akt activation [3,4].

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Previously, the Agazie group demonstrated that Shp2 knockdown reduces anchorage-independent growth of breast cancer cells and promotes acquisition of an epithelial phenotype [9], but whether Shp2 regulated breast TICs was unclear. Aceto and colleagues address this question using conditional, miRNA-adapted shRNA-mediated knockdown of Shp2 in a variety of HER2 or triple-negative breast cancer cell lines, as well as MCF-10A mammary epithelial cells engineered to overexpress HER2 and HER3. Shp2 knockdown attenuated invasion *in vitro* and *in vivo*, blocked growth of orthotopic tumour xenografts, and reduced lung metastasis [2]. Moreover, Shp2 knockdown reduced the self-renewal capacity of tumoursphere-forming cells as well as the number of cells exhibiting a CSC phenotype. Single cells isolated from Shp2-deficient tumours exhibited a reduced ability to re-seed new tumours, providing strong evidence that Shp2 is required for the maintenance and propagation of TICs.

Given the potent biological effects of Shp2 ablation, a critical question was the nature of the signalling and transcriptional networks regulated by this phosphatase. The data presented indicate that Erk plays a key role downstream of Shp2 [2], but other pathways probably also contribute. In addition, transcript profiling identified an Shp2 signature of 180 genes that exhibited reduced expression upon Shp2 knockdown. This included the genes encoding zinc finger E-box binding homeobox 1, a transcription factor that induces epithelial-to-mesenchyme transition, and the c-Myc target lin-28 homolog B, a repressor of Let-7 miRNA biogenesis. Consistent with these data, Shp2 knockdown resulted in increased expression of let-7a and let-7b, and downregulation of the let-7 targets Ras and c-Myc. Since let-7 targets include proteins upstream of Shp2, these studies identify a novel positive-acting feedback loop that regulates maintenance of TICs.

Interestingly, a previous paper from the Struhl laboratory demonstrated that transient Src signalling sets up a self-reinforcing feedback loop for CSC maintenance in which NF-κB activates lin-28 homolog B to repress Let-7 processing, which then leads to IL-6 expression [10], highlighting the importance of positive feedback loops in

maintaining this cellular phenotype. It would be interesting to determine the role of the miR-200 family of miRNAs in this system, given that miR-200 forms a double-negative regulatory loop with zinc finger E-box binding homeobox 1 and thereby represses epithelial-to-mesenchyme transition and the CSC phenotype [11].

While Shp2 was not consistently overexpressed in breast cancer compared with normal breast tissue, the Shp2 signature was enriched in the triple-negative subtype versus the luminal or HER2 breast cancer subtypes, in invasive cancers versus ductal carcinoma *in situ*, and in poor-prognosis breast cancers. While this enrichment might be indicative of Shp2 activation, it should be noted that other pathways are likely to converge on this gene set, and the expression pattern might reflect intrinsic properties of particular breast cancer phenotypes rather than a role for Shp2 *per se*. However, the authors present a strong case that Shp2 represents a potential therapeutic target in at least a subset of HER2 and triple-negative breast cancers, two breast cancer subtypes often associated with a poor prognosis [12].

A major issue in the field of CSC biology has been the role that phenotypic plasticity plays in acquisition of a stem-like phenotype, with some data suggesting that all cancer cells have the capacity to acquire this state in response to environmental cues [1]. If this is true, then therapeutics designed to target CSCs may not lead to durable clinical responses in the adjuvant setting. A highlight of this work is the rigorous investigation of the TIC phenotype following Shp2 knockdown, in which the authors show not only quantitative inhibition of tumour growth and tumour-initiating capacity, but also the failure to reacquire the stem-like state after release from Shp2 knockdown – suggesting that in these models depletion of Shp2 leads to irreversible loss of stem-like capacity. A key implication of these findings is that components of the identified positive feedback loop represent potential therapeutic targets, and in this context it is worth noting that small-molecule inhibitors of Shp2 are currently in preclinical development [13]. In addition, characterisation of the upstream regulator(s) of Shp2 in the TICs may identify additional targets, such as specific receptor tyrosine kinases, which are amenable to inhibitor-based or antibody-based therapies.

Abbreviations

CSC, cancer stem cell; IL, interleukin; miRNA, microRNA; NF, nuclear factor; Shp2, Src homology 2 domain-containing protein tyrosine phosphatase 2; shRNA, short hairpin RNA; TIC, tumour-initiating cell.

Competing interests

The authors declare that they have no competing interests.

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